- Dr
- c. secreting of the monoclonal antibodies in a hybridoma cell culture and isolation of the monoclonal antibodies from it or production of the monoclonal antibodies by injection of the hybridoma cells into animals, and isolation of the monoclonal antibodies from the body fluid of the animals.
- 3. (Amended) A monoclonal antibody according to claim 1, with the hybridoma cells enabled to produce monoclonal human-CD28 specific animal antibodies being available through
  - a) creation of a plasmid by means of insertion of human-CD28 cDNA into the pHβAPr-1-neo vector following excision of the SaII-HindIII fragment and production of protoplasts from Escherichia coli (MC1061) which carry the plasmid,
  - b) fusing of the protoplasts with mouse A20J and/or L929 tumour cells using polyethylene glycol,
  - c) cultivation of the transfected cells received in b) above,
  - d) screening of the transfected mouse A20J and/or L929 cells for the expression of human CD28 and selection of mouse A20J and/or L929 cells expressing human-CD28,
  - e) immunization of BALB/c mice with mouse A20J and/or L929 cells expressing human-CD28,
  - removal of spleen cells of the mice immunized in this way and fusing the spleen cells with cells of the cell line  $\chi$ 63-Ag 8.653 by means of polyethylene glycol,
  - g) selection of the hybridoma cells received in this way with the condition that in the supernatant of selected hybridoma cells there are antibodies contained which bind on human CD28 expressing mouse A20J and/or L929 cells, and
  - h) cultivation/sub-cloning of the selected hybridoma cells obtained in g) above and isolating the monoclonal antibodies.
  - 4. (Amended) A hybridoma cell for the production of a monoclonal antibody according to claim 1, which is available through the following:

- a) creation of a plasmid by means of insertion of human-CD28 cDNA into the pHβAPr-1-neo vector following excision of the SaII-HindIII fragment and production of protoplasts from Escherichia coli (MC1061) which carry the plasmid,
  - fusing of the protoplasts with mouse A20J and/or L929 tumour cells using polyethylene glycol,
  - cultivation of the transfected cells received in b) above,
    - screening of the transfected mouse A20J and/or L929 cells for the expression of human CD28 and selection of mouse A20J and/or L929 cells expressing human-CD28,
- e) immunization of BALB/c mice with mouse A20J and/or L929 cells expressing human-CD28,
- f) removal of spleen cells of the mice immunized in this way and fusing the spleen cells with cells of the cell line X63-Ag 8.653 by means of polyethylene glycol, and
- g) selection of the hybridoma cells received in this way with the condition that in the supernatant of selected hybridoma cells there are antibodies contained which bind on human CD28 expressing mouse A20J and/or L929 cells.

13. (Amended) A monoclonal antibody according to claim 2, enabled to produce monoclonal human-CD28 specific animal antibodies being available through

- a) creation of a plasmid by means of insertion of human-CD28 cDNA into the pHβAPr-1-neo vector following excision of the SaII-HindIII fragment and production of protoplasts from Escherichia coli (MC1061) which carry the plasmid,
- b) fusing of the protoplasts with mouse A20J and/or L929 tumour cells using polyethylene glycol,
- c) cultivation of the transfected cells received in b) above,
- d) screening of the transfected mouse A20J and/or L929 cells for the expression of human CD28 and selection of mouse A20J and/or L929 cells expressing human-CD28,

b)

c)

d)

G.

- e) immunization of BALB/c mice with mouse A20J and/or L929 cells expressing human-CD28,
- f) removal of spleen cells of the mice immunized in this way and fusing the spleen cells with cells of the cell line X63-Ag 8.653 by means of polyethylene glycol,
- g) selection of the hybridoma cells received in this way with the condition that in the supernatant of selected hybridoma cells there are antibodies contained which bind human CD28 expressing mouse A20J and/or L929 cells, and
- h) cultivation/sub-cloning of the selected hybridoma cells obtained in g) above and isolating of the antibodies therefrom.
- 14. (Amended) A hybridoma cell for the production of a monoclonal antibody according to claim 2 which is available through the following:
  - a) creation of a plasmid by means of insertion of human-CD28 cDNA into the pHβAPr-1-neo vector following excision of the SaII-HindIII fragment and production of protoplasts from Escherichia coli (MC1061) which carry the plasmid,
  - b) fusing the protoplasts with mouse A20J and/or L929 tumour cells using polyethylene glycol,
  - c) cultivation of the transfected cells received in b) above,
  - d) screening of the transfected mouse A20J and/or L929 cells for the expression of human CD28 and selection of mouse A20J and/or L929 cells expressing human-CD28,
  - e) immunization of BALB/c mice with mouse A20J and or L929 cells expressing human-CD28,
  - f) removal of spleen cells of the mice immunized in this way and fusing the spleen cells with cells of the cell line X63-Ag 8.653 using polyethylene glycol, and
  - g) selection of the hybridoma cells received in this way with the condition that in the supernatant of selected hybridoma cells there are antibodies contained which bind human CD28 expressing mouse A20J and/or L929 cells.

- 15. (Amended) A hybridoma cell for the production of a monoclonal antibody according to claim 3 which is available through the following:
  - a) creation of a plasmid by means of insertion of human-CD28 cDNA into the pHβAPr1-neo vector following excision of the SaII-HindIII fragment and production of
    protoplasts from Escherichia coli (MC1061) which carry the plasmid,
  - b) fusing of the protoplasts with mouse A20J and/or L929 tumour cells using polyethylene glycol,
  - c) cultivation of the transfected cells received in b) above,
  - d) screening of the transfected mouse A20J and/or L929 cells for the expression of human CD28 and selection of mouse A20J and/or L929 cells expressing human-CD28,
  - e) immunization of BALB/c mice with mouse A20J and/or L929 cells expressing human-CD28,
  - f) removal of spleen cells of the mice immunized in this way and fusing the spleen cells with cells of the cell line X63-Ag 8.653 using polyethylene glycol, and
  - g) selection of the hybridoma cells received in this way with the condition that in the supernatant of selected hybrdoma cells there are antibodies contained which bind human CD28 expressing mouse A20J and/or L929 cells.
- 24. (Amended) A hybridoma cell for the production of a monoclonal antibody according to claim 13 which is available through the following:
  - a) creation of a plasmid by means of insertion of human-CD28 cDNA into the pHβAPr-1-neo vector following excision of the SaII-HindIII fragment and production of protoplasts from Escherichia coli (MC1061) which carry the plasmid,
  - b) fusing of the protoplasts with mouse A20J and/or L929 tumour cells using polyethylene glycol,
  - c) cultivation of the transfected cells received in b) above,

only,

D3-91 e)

screening of the transfected mouse A20J and/or L929 cells for the expression of human CD28 and selection of mouse A20J and/or L929 cells expressing human-CD28,

- immunization of BALB/c mice with mouse A20J and/or L929 cells expressing human-CD28,
- f) removal of spleen cells of the mice immunized in this way and fusing the spleen cells with cells of the cell line X63-Ag 8.633 using polyethylene glycol, and
- g) selection of the hybridoma cells received in this way with the condition that in the supernatant of selected hybridoma cells there are antibodies contained which bind human CD28 expressing mouse A20J and/or L929 cells.

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